Channel Regulation & Modulation I

660-Pos Board B446

ICA105574 Binds to a Similar Region of the Pore Domain to Induce Opposite Effects on the Gating of EAG and ERG Channels

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ICA activates hERG1 channels by reducing inactivation, but inhibits hEAG1 channels by inducing inactivation. Molecular modeling suggests that ICA binds to a pocket located between S5 and S6 in both channels. A mutation in S5 (F557L) or S6 (Y652A) of hERG1 eliminates the activity of ICA, indicating a key role of these residues in the drug binding site (Garg, et al 2011, Mol. Pharm. 80: 630). The equivalent mutations in hEAG1 lead to very different effects. First, F359L hEAG1 channels are activated by ICA (opposite to effects on wild-type channels). Second, Y464A induces inactivation of hEAG1 channels, an effect accentuated by ICA. The combined mutation (Y464A/F359L) in hEAG1 eliminates inactivation, and prevents activation of channels by ICA. These findings suggest that both Y464 and F359 residues, and perhaps their interaction, are important for intrinsic and ICA-modulated inactivation of hEAG1 channels.

661-Pos Board B447

Modulation of EAG Channel Inactivation by ICA105574

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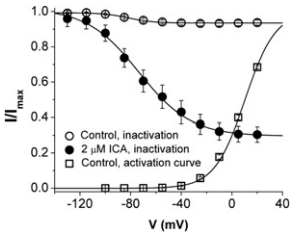
Ether-a-go-go (EAG) family potassium channels, including hEAG1 and hERG1 play an important role in the heart, CNS, cell cycle regulation and cancer. ICA-105574 (ICA) was previously reported to activate hERG1 current by causing a large depolarizing shift in the $V_{0.5}$ for P-type inactivation (Gerlach et al, *Mol. Pharmacol.* 2010, 77: 58). The related hEAG1 channel exhibits little or no inactivation; however, in the presence of ICA (2 μM) channels exhibit pronounced inactivation ($V_{0.5} = -74$ mV). Voltage dependence of inactivation and activation for WT hEAG1 with and without ICA is shown in the figure. At 10 μM ICA, inactivation reduces maximum current by >80%. Mutation of a single Tyr residue in S6 (Y464A) of hEAG1 also induces prominent inactivation which is accentuated by ICA. Combined mutation of two residues located near the selectivity filter (T432S and A443S) induces a rapid P-type inactivation similar to hERG1 that is removed by ICA. Modulation of inactivation by ICA can result in either activation or inhibition of EAG1/ERG1 channels.



662-Pos Board B448

The Acceleration of the Inactivation of Prokaryotic Sodium Channel by the Formation of C-Terminal 4-Helix Bundle

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Voltage-gated sodium channels play essential roles in many important physiological processes, including electric signalling and muscle contraction. Therefore their activation and inactivation are needed to be strictly regulated.

Here, we show that the cytosolic C-terminal region of NavSulP, a prokaryotic voltage-gated sodium channel (NavBac) cloned from *Sulfitobacter pontiacus*, accelerates C-type inactivation. The crystal structure of the C-terminal region of NavSulP in a NaK-NavSulP chimera channel at 3.2 Å resolution revealed that the C-terminal region forms a four-helix bundle. Point mutations of the residues of the four-helix bundle, which were involved in intersubunit interactions, destabilised the tetramer of the channel and reduced the inactivation rate. This result suggested that the formation of the four-helix bundle accelerates the inactivation of NavSulP. NavBac inactivation seems to be regulated only by the C-type inactivation, which is thought to be due to collapse of the selectivity filter through a conformational change of the activation gate of the inner helix of the pore domain. The C-terminal four-helix bundle is connected to the inner helix. The increase of the rigidity of the inner helix with the glycine-to-alanine mutation also reduced the inactivation rate of NavSulP as well as the destabilisation of the four-helix bundle. On the other hand, the glycine-to-alanine mutation did not destabilize the formation of tetrameric channel, and then the C-terminal region could maintain the four-helix bundle. It was thought that the rigidity of inner helix disabled the formation of C-terminal four-helix bundle from accelerating the C-type inactivation rate.

These findings suggest that the formation of four-helix bundle of NavSulP plays important roles not only in stabilising the tetramer, but also in accelerating C-type inactivation by promoting a conformational change of the inner helices of the pore domain.

663-Pos Board B449

Detailed Examination of TRPM7 Channel Mg^{2+} Dependence

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TRPM7, a member of transient receptor potential superfamily of ion channels, underlies the Mg^{2+}-inhibited cation currents highly expressed in various cell types of hematopoietic lineage. TRPM7 channels are sensitive to intracellular as well as extracellular Mg^{2+} and other polyvalent cations. The present study was undertaken to characterize the inhibition of TRPM7 channels by intracellular Mg^{2+} in detail. In order to generate a dose-response curve for $Mg^{2+}{}_i$, we systematically varied the internal Mg^{2+} concentration and measured the corresponding maximal TRPM7 current amplitude with whole-cell patch clamp in Jurkat T lymphocytes and HEK293 cells. Mg^{2+}-containing solutions were buffered with HEDTA. We tested free Mg^{2+} concentrations of 100 nM to 400 μM in $n \geq 70$ cells. Unexpectedly, we find that the dose-response curve for Mg^{2+} is biphasic, yielding IC_{50} values of ~8 μM and ~200 μM. 300 μM free Mg^{2+} is sufficient for complete inhibition of channel activity. This finding suggests the existence of *two inhibitor sites* for TRPM7 activity. Since in whole-cell configuration the channel interior is only exposed to one solution throughout the recording, it is impossible to separate Mg^{2+} effect on the likelihood of channel opening from its effect on an already open channel. Moreover, reversible inhibition cannot be distinguished from time-dependent rundown of activity. In order to examine the nature of Mg^{2+}-mediated inhibition more thoroughly, we recorded TRPM7 channels in inside-out patch configuration. Membrane patches were excised into a Mg^{2+}-free bath solution and TRPM7 single-channel activity recorded. Mg^{2+} was then added to the solution facing intracellular side of the channel, using rapid application. We observed reversible inhibition by Mg^{2+} in the 35 - 400 micromolar concentration range and have characterized changes in single-channel characteristics responsible for the inhibition. A comparison of Mg^{2+} and proton sensitivities is also presented.

664-Pos Board B450

M-Type K^+ Channel Openers: In Vivo Neuroprotective Role During Cerebrovascular Stroke

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Investigations of neuroprotection during ischemic stroke through ''M-channels'' could underlie potential treatments, since K^+ channels stabilize resting potentials by counterbalancing the depolarizing effects of excitatory cation currents. Produced by combinations of KCNQ2-5 subunits, these voltage-gated channels, with distinct electrophysiological and pharmacological properties, play critical roles in control of neuronal excitability and action potential firing. A known M-channel activator, retigabine (RTG) and other novel openers, such as NH29 and QO-58, up-regulate M-channels by stabilizing their open states through distinct molecular mechanisms. We have previously shown M-channels to be up-regulated by reactive oxygen species (ROS), molecules commonly produced during and after ischemic cerebrovascular stroke. We hypothesize that M current-mediated neuronal silencing has a neuroprotective role by increasing opening of KCNQ channels, thus decreasing neuronal activity and prolonging activation of cellular cascades that can prevent cell death. Furthermore, we hypothesize that drugs that shift voltage-dependence of M-channels toward negative potentials and increase opening, will significantly reduce the severity of the ischemic lesion following cerebrovascular stroke. We used two *in vivo* mouse models, the first a cerebral infarct produced by laser-controlled photothrombosis and the second the middle cerebral artery occlusion (MCAo). Whereas the photothrombosis causes transient ischemic attacks in the parietal cortex, the MCAo model produces a catastrophic stroke in the **ipsilateral** hemisphere. M-current activity was pharmacologically altered in both models using RTG, NH29 and QO-58 and the blocker, XE991. In control, blood vessel photothrombosis caused cell death over a 24-hour period. Interestingly, application of M-channel activators significantly reduced lesion area, whereas XE991 exacerbated the area of cell death. Thus, this study uses powerful models which may provide novel therapeutics for commonly-occurring ischemic attacks. We will also show the results of in vivo imaging and mouse behavioral assays that correlate with histological data.